

Original Article

Three Case Reports to Illustrate Clinical Applications in the Use of Erythrocyte Transketolase

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Non-caloric nutrients (NCN) are extremely numerous and it is more than obvious that they work in a team relationship. These vitally important interactions are, for the most part, poorly understood. These brief case reports illustrate this in the therapeutic use of thiamin in a clinical setting. The initially abnormal erythrocyte transketolase activity (TKA) and/or the thiamin pyrophosphate effect (TPPE), indicating intracellular cofactor deficiency, usually improves with thiamin administration. Biochemical correction of the abnormality is, however, invariably dependent on the provision of other NCN, especially magnesium. In two patients reported here, this correction required several infusions containing magnesium and other NCN administered intravenously. In a third patient, hemoconcentration associated with an abnormal TPPE was normalized after administration of nutrients that included thiamin and magnesium.

Keywords: transketolase – thiamin/magnesium deficiency – hemoconcentration

Introduction

All the patients in these case reports were found to have evidence of intracellular thiamin deficiency by means of erythrocyte transketolase studies. This was demonstrated by an abnormally decreased transketolase activity (TKA), an increased thiamin pyrophosphate effect (TPPE), or both together. Experience with the administration of thiamin or thiamin tetrahydrofurfuryl disulfide (TTFD) in patients has shown that it is usually easy to correct abnormal TKA/TPPE studies and that it correlates with clinical improvement (1–4). In two of the three patients reported here, TKA/TPPE studies became worse in spite of large doses of thiamin and magnesium given by mouth. Correction of the biochemical abnormality was not achieved until nutrients were given by intravenous infusion. In a third patient, symptoms were accompanied by an abnormal TPPE and hemoconcentration, both of which became normal with the administration of TTFD.

Methods

Not many laboratory tests for nutritional deficiency are performed that depend on enzymatic function. Transketolase is an enzyme that occurs twice in the hexose monophosphate shunt and its activity depends on thiamin and magnesium as cofactors. Because this biochemical pathway occurs in erythrocytes, the test can be performed on a blood sample (5,6). It first depends on measuring the baseline speed of synthesis of the reaction product, sedulose-7-phosphate and this is reported as TKA in units per liter of blood per minute. The normal laboratory range for TKA is 42–86 mU min⁻¹. The second part of the test depends on the *in vitro* addition of thiamin pyrophosphate (TPP), after controlling for magnesium, and measuring the reaction again. If the synthesis of the enzyme product accelerates it is reported as the percentage increase over the baseline rate and referred to as the TPPE. The normal laboratory range for this is 0–17%.

Case Reports

Case 1

J.S. was a 77-year-old woman. In June 2005, she came to our clinic complaining of extreme fatigue. This was severe enough

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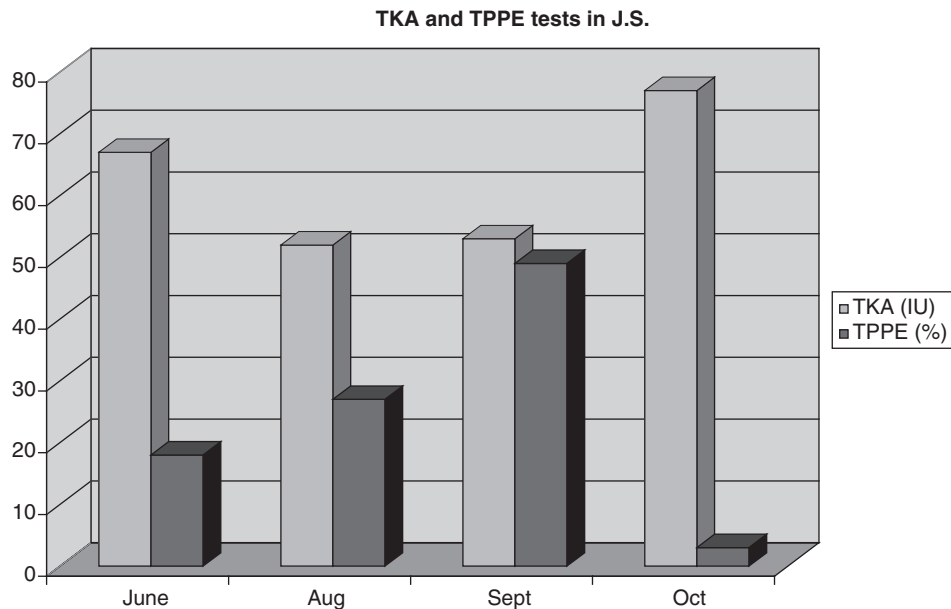


Figure 1. TKA and TPPE tests in J.S.

to cause her to go to sleep briefly while driving. She was also experiencing occasional 'dizziness' on rising in the morning and severe constipation. She reported that an ophthalmologist had found increased pressure in one eye and she was using eye drops prescribed for this. Past history revealed that she had received radioactive iodine as a child and was being treated with synthetic T3. Although the TKA was in the normal range (Fig. 1) the TPPE was 18%, indicating a mild degree of thiamin deficiency.

She was treated with orally administered nutritional supplements that included a multivitamin, 3 g of ascorbic acid (bowel tolerance), 300 mg of magnesium/potassium/aspartate, 250 mg of calcium with 166 mg magnesium in a combination tablet taken at bedtime, 5 mg of phytonadione (vitamin K1) because of a history of osteoporosis diagnosed elsewhere, 200 mg of lipoic acid and 150 mg of TTFD.

Two months later she reported that she had 'more energy'. In spite of this the TKA was lower in concentration and the TPPE had accelerated dramatically (Fig. 1). Although there was no exacerbation of symptoms, 1 month later the laboratory results had deteriorated again and it was decided to provide her with a series of intravenous infusions (Table 1). Following this treatment, the laboratory test was repeated. The TKA had increased its activity and the TPPE had fallen to 3% (Fig. 1)

Case 2

F.G. was first seen at the age of 39 years with what he described as 'easily pulled muscles on exercise' and fatigue. A constant complaint over the years was carpo pedal spasms and occasional hypogastric discomfort, also often associated with exercise. With a recent office visit, at the age of 56 years he admitted to constant dietary indiscretions with simple carbohydrates. Because of an abnormal TPPE (Fig. 2) the nutrients

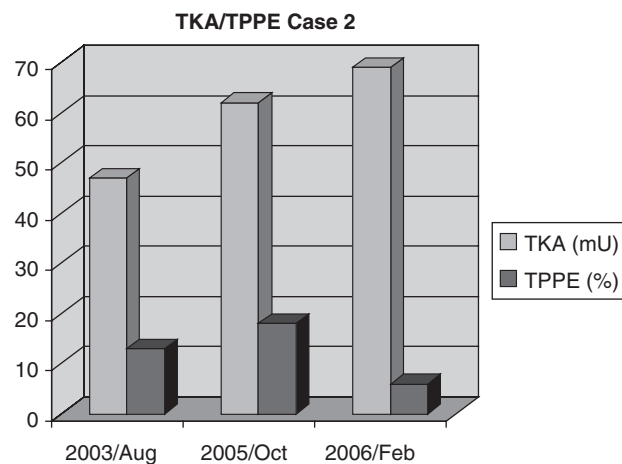


Figure 2. TKA/TPPE Case 2.

Table 1. Contents of the intravenous infusions given to J.S.

Sodium chloride	0.9%	250 ml
Magnesium chloride	2 g	10 ml
Calcium gluconate	10%	10 ml
Pyridoxine	100 mg	1 ml
Dexpanthenol	1 g	4 ml
Hydroxocobalamin	1 mg	1 ml
Ascorbic acid	6 g	12 ml

provided included TTFD and magnesium/potassium/aspartate, p.o. He was seen again 2 months later and there had been no change in symptoms. The TPPE had increased although TKA had improved in activity (Fig. 2). Between January 10 and 16 he received four of the same nutrient infusions given to the

Table 2. Laboratory studies: Case 3

	TKA (mu)	TPPE (%)	RBC (mill mcl ⁻¹)	Hb (g dl ⁻¹)	Hematocrit (%)
September	46	33	5.36	15.7	46.1
November	77	3	4.5	14	41.3

patient in Case 1 (Table 1). During this period of administering the infusions, his symptoms disappeared, but began to return after their completion. The TKA had again increased and the TPPE had decreased into the acceptable range of normal.

Case 3

J.K. This 72-year-old woman was first seen because of 'flu-like' symptoms that occurred every 2 weeks after square dancing. She would feel chilled, experience nausea and become unusually fatigued. Low-grade fever would last about 4 days. She would feel well again until after the next session of dancing. She reported recurrent sinus infections that had occurred over a number of years, each being treated with an antibiotic.

Laboratory studies are shown in Table 2 before and after 2 months of nutrient therapy. Dietary counseling and supplementation correlated with clinical improvement. Supplements included a multivitamin as a nutritional foundation, up to 3 g of ascorbic acid, depending on bowel tolerance, 240 mg EPA, 60 mg GLA, B complex with 50 mg additional pyridox-5-phosphate, 270 mg magnesium and 270 mg of potassium as magnesium/potassium/aspartate and 150 mg TFFD.

Discussion

Theoretically, the transketolase enzyme should be saturated with TPP and there should be no acceleration of its activity after the addition of TPP. The percentage acceleration should be thought of as a gradual transition from thiamin sufficiency to deficiency. The result of the test is at its worst when the TKA is below the laboratory range and the TPPE is increased over the acceptable range of 17%. Moderate deficiency may be recorded with a TKA in the normal laboratory range and an abnormal increase in TPPE. Although the test is usually used exclusively for evidence of intracellular thiamin deficiency (3–6), an increased activity of TKA was reported as being able to distinguish B12-deficient from folate-deficient pernicious anemia (7). It also should be possible to use the test for magnesium deficiency by controlling for TPP, but this has never been done to my knowledge.

It has long been known that magnesium and thiamin have a close biochemical relationship. Itokawa *et al.* (8) found that tissue TKA decreased markedly in thiamin-deficient (TD) and thiamin–magnesium-deficient (TMD) rats. Addition of TPP to tissue homogenates of TD rats resulted in recovery of TKA. This did not occur when rats were also deficient in magnesium. In subcellular fractions of the liver of magnesium-deficient rats thiamin content was most markedly decreased in the

mitochondrial fraction (9). Fujiwara *et al.* (10) showed that both calcium and magnesium are important components of a diet aimed at providing adequate thiamin nutrition. Jung *et al.* (11) studied the binding kinetics for [³⁵S] TPP to transketolase and the dependency of the enzyme on divalent cations for activity. They showed that transketolase contained Mg²⁺ in its molecular structure.

Itokawa *et al.* (12) suggested that excess thiamine promotes magnesium deficiency and liberates serotonin into the blood stream from mast cells. In thiamin-excess with magnesium deficiency and in thiamine-adequate, magnesium-deficient rats, serotonin oxidation activity in liver and stomach decreased significantly, while this decrease was not observed in thiamine-deficient, magnesium-deficient rats (13). Liver cholesterol and lipid levels in thiamine-adequate magnesium-deficient and in thiamine-excess magnesium-deficient rats increased significantly (14). Basic science studies indicate that a physiological concentration of Mg²⁺ can regulate TPP binding to the thiA riboswitch (15). Coenzymes exert their catalytic activity after binding to a specific protein component, another variable in showing correction of TKA abnormalities (16). Although the free coenzyme TPP can assist some of its coenzyme reaction, the protein environment potentially accelerates the overall enzyme reaction by up to a factor of 10¹² (17). Thus, there may be genetically determined factors that complicate thiamin therapy.

The intimate relationship of thiamin and magnesium was demonstrated clinically in a patient with Crohn's disease. Long-standing diarrhea resulted in combined thiamin and magnesium deficiency. Despite massive doses of thiamin administered intravenously, the symptoms of thiamin deficiency could not be relieved until correction of the magnesium deficiency (18). Wernicke encephalopathy, a well known result of thiamin deficiency, has been reported as being induced by magnesium deficiency (19).

Fifty patients with abnormal transketolase activity coefficient (ETK-AC) and affinity for coenzyme (Km-TPP) had associated fibromyalgia or senile dementia of Alzheimer type (20). Compared with 12 untreated patients, ETK-AC was significantly decreased with administration of thiamin and pyridoxine. The Km-TPP was significantly decreased with high energy phosphates or piracetam. In 9 other patients treated with a combination of high energy phosphates, vitamins B1, B6, and magnesium, ETK-AC and Km-TPP were both significantly decreased.

It is to be noted that the patient J.S. (Case 1) had been found to have increased pressure in one eye and was being treated with eye drops by an ophthalmologist. Evidence exists that autonomic nervous system dysfunction is present in open angle glaucoma, resulting in disturbed autoregulation, leading to vascular dysregulation in the eye (21,22). Cold provocation in patients with this form of glaucoma produced different blood pressure responses and ocular blood flow response as compared with controls (23), suggesting that environmental stress might play a part. Thiamin deficiency has long been known to cause autonomic nervous system dysfunction (24).

She was receiving large doses of magnesium and other nutritional supplements by mouth while the TKA/TPPE results became worse. They improved rapidly into the normal range after magnesium, with other non-caloric nutrients (NCN), was given intravenously. Ascorbic acid has been shown to activate thiamin diphosphatase in rat brain (25) The studies discussed here show that magnesium is of vital importance in the normal TKA/TPPE combination, but some of them suggest that TKA is dependent on a number of nutrient variables. Also, this laboratory test only shows a particular aspect of thiamin metabolism and does nothing to expose a failure to synthesize thiamin triphosphate, so important in the central nervous system (26).

The hemoconcentration associated with thiamin deficiency in the patient in Case 3 requires comment. Erythrocytosis has been observed in TD rats (27). The number of reticulocytes and plasma erythropoietin levels increased and the levels of 2,3-diphosphoglycerate of RBCs decreased. A Medline search failed to reveal any similar observation in humans. Oxygen-chemosensitive sites are distributed throughout the brainstem from the thalamus to the medulla and may form an oxygen-chemosensitive network (28), although these authors emphasized that little is known regarding the cellular mechanisms involved in the chemotransduction process of the central oxygen sensors.

Experimentally induced hypoxia in rats induced polycythemia (29), a phenomenon that is usually considered as a compensatory effect. Increase in erythropoietin appears to be the mechanism (30).

Although the referenced research is related to the study of high-altitude compensatory physiology, it is possible that thiamin deficiency might imitate high altitude by its effect on brainstem. The recurrent 'flu-like' symptoms experienced by JS after physical activity might have been the equivalent of recurrent mountain sickness. Adaptation at the cellular level involves a shift from oxidative phosphorylation to anaerobic glycolysis, increased glucose metabolism and expression of hypoxic stress-related proteins. Thiamin biochemistry, metabolism and pathophysiology was recently reviewed (31). It was pointed out that the role of the vitamin in brain function is still incompletely understood, particularly in the form of thiamin triphosphate. Until it becomes possible to detect this vital component by clinical laboratories, its deficiency cannot be detected easily.

References

- Lonsdale D, Shamberger RJ. The potential value of red cell transketolase in the metabolic evaluation of disease. *Cleve Clin Quart* 1978;45:267-74.
- Schwartz RA, Gross M, Lonsdale D, Shamberger RJ. Transketolase activity in psychiatric patients. *J Clin Psychiatry* 1979;40:34-6.
- Lonsdale D, Shamberger RJ. Red cell transketolase as an indicator of nutritional deficiency. *Am J Clin Nutr* 1980;33:205-11.
- Lonsdale D. Red cell transketolase studies in a private practice specializing in nutritional correction. *J Am Coll Nutr* 1988;7:61-7.
- Brin M, Tai M, Ostashever AS, Kalinsky H. Effect of thiamine deficiency on activity of erythrocyte-hemolysate transketolase. *J Nutr* 1960;71:273-80.
- Massod MF, McGuire SL, Werner KR. Analysis of blood transketolase activity. *Am J Clin Pathol* 1971;55:465-70.
- Markkannen T, Kalliomaki JL. Transketolase activity of blood cells in various clinical conditions. *Am J Med Sci* 1966;252:564-9.
- Itokawa Y, Inoue K, Natori Y, Okazaki K, Fujiwara M. Effect of thiamine on growth, tissue magnesium, thiamine levels and transketolase activity in magnesium deficient rats. *J Vitaminol* 1972;18:159-64.
- Itokawa Y, Tseng L-F, Fujiwara M. Thiamine metabolism in magnesium deficient rats. *J Nutr Sci Vitaminol* 1974;20:249-55.
- Fujiwara M, Itokawa Y, Kimura M. Experimental studies on the relationships between thiamine and divalent cations, calcium and magnesium. In: Gubler CJ, Fujiwara M, Dreyfus PM (eds). *Thiamine*. NY: John Wiley and Sons, 1976, 63-80.
- Jung EH, Takeuchi T, Nishino K, Itokawa Y. Studies on the nature of thiamine pyrophosphate binding and dependency on divalent cations of transketolase from human erythrocytes. *Int J Biochem* 1988;20:1255-9.
- Itokawa Y, Tanaka C, Kimura M. Effect of thiamine on serotonin levels in magnesium-deficient animals. *Metabolism* 1972;21:375-9.
- Itokawa Y, Kimura M, Inagaki C, Tanaka C. Effect of thiamine on serotoninoxidation in magnesium-deficient rats. *J Nutr Sci Vitaminol* 1974;20:27-33.
- Itokawa Y, Sasagawa S, Fujiwara M. Effects of thiamine on lipid metabolism in magnesium deficient rats. *J Nutr Sci Vitaminol* 1973;19:15-21.
- Yamauchi T, Miyoshi D, Kubodera T, Nishimura A, Nakai S, Sugimoto N. Roles of Mg²⁺ in TPP-dependent riboswitch. *FEBS Lett* 2005;579:2583-8.
- Said HM. Recent advances in carrier-mediated intestinal absorption of water soluble vitamins. *Annu Rev Physiol* 2004;66:419-46.
- Kern D, Kern G, Neef H, Tittmann K, Killenberg-Jabs M, Wikner C. How thiamine diphosphate is activated in enzymes. *Science* 1997;275:67-70.
- Dyckner T, Elk B, Nyhlin H, Wester PO. Aggravation of thiamine deficiency by magnesium depletion. A case report. *Acta Med Scand* 1985;218:129-31.
- McLean J, Manchip S. Wernicke's encephalopathy induced by magnesium depletion. *Lancet* 1999;353:1768.
- Eisinger J, Bagneres D, Arroyo P, Plantamura A, Ayavou T. Effects of magnesium, high energy phosphates, piracetam and thiamin on erythrocyte transketolase. *Magnes Res* 1994;7:59-61.
- Kumar R, Ahuja VM. Glaucoma and concomitant-status of autonomic nervous system. *Indian J Physiol Pharmacol* 1998;42:90-4.
- Grieshaber MC, Flammer J. Blood flow in glaucoma. *Curr Opin Ophthalmol* 2005;16:79-83.
- Ghergel D, Hosking SL, Cunliffe LA. Abnormal systemic and ocular vascular response to temperature provocation in primary open-angle glaucoma patient: a case for autonomic failure? *Invest Ophthalmol Vis Sci* 2004;45:3546-54.
- Inouye K, Katsura E. Etiology and pathology of beriberi. In: Shimazono N, Katsura E (eds). *Beriberi and Thiamine*. Tokyo: Igaku Shoin Ltd, 1965, 1-28.
- Matsuda T, Maeda S, Baba A, Iwata H. Existence of ascorbic acid as an activator of thiamine diphosphatase in rat brain. *J Neurochem* 1979;32:443-8.
- Iwata H, Baba A, Matsuda T, Terashita Z, Ishii K. Role of thiamine metabolism in the central nervous system II. Effects of various agents on thiamine triphosphatase activity in rat brain. *Jpn J Pharmacol* 1974;24:825-9.
- Hobara R, Yasuhara H. Erythrocytosis in thiamine deficient rats. *Jpn J Pharmacol* 1981;31:985-93.
- Neubauer JA, Sunderram J. Oxygen-sensing neurons in the central nervous system. *J Appl Physiol* 2004;96:367-74.
- Zucali JR, Lee M, Mirand EA. Carbon dioxide effects on erythropoietin and erythropoiesis. *J Lab Clin Med* 1978;92:648-55.
- Bozzini CE, Barcela AC, Conti MI, Martinez MP, Alippi RM. Enhanced erythropoietin production during hypobaric hypoxia in mice under treatment to keep erythrocyte mass from rising: implications for the adaptive role of polycythemia. *High Alt Med Biol* 2005;6:238-46.
- Lonsdale D. A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. *Evid Based Complement Alternat Med* 2006;3:49-59.